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Using zebrafish to understand the genome: an interview with Nancy Hopkins

Nancy Hopkins is famous for pushing the envelope: both in her pursuit to understand cancer genetics and in exposing professional disparities between men and women. Here, she discusses influential moments that have defined her career path, and her concerns for the future.

To unlock the valuable secrets in our genetic sequence, scientists are working to define the independent and coordinated roles of an overwhelming number of genes. Nancy Hopkins offers an innovative approach to the problem by adapting old methodologies to the unlikely, but tractable, zebrafish model. She uses mouse retroviral vectors to create genetic mutations in the fish, which are tagged to allow easy and precise cloning. To date, her forward genetic screens have resulted in the isolation and cloning of over 275 unique genes that have important roles in development or cancer predisposition. Almost all of these genes have human homologues.

Despite her prestigious awards, including her membership to the US National Academy of Sciences, and her successful career as a professor at MIT, it has not always been a smooth journey for Nancy Hopkins. She recently shared with us some of her story about her perseverance through good times and bad.

You made a daring leap from your early work on cancer to genetic screening in zebrafish. How did you decide to make such a bold move?

When I was very young, I decided to go into science after hearing Jim Watson give a lecture on molecular biology. The year was 1963, a year when the genetic code was still being cracked. I was an undergraduate in Radcliff, then the girl's division of Harvard. After that 1-hour lecture I thought that this new field of molecular biology was going to answer all of the profound questions that really plagued people. When you are young, you ask questions like: What is the meaning of life? And I thought listening to Jim, 'Wow, this is about the meaning of life. Molecular biology is going to explain the secret to life. This is as close to understanding life as I will ever get.' There were two things I was passionately interested in that I thought molecular biology might one day be able to address. One was the basis of cancer and the other was the basis of the brain and how it works. In that era, we didn't really think it would be possible to apply molecular biology to those fields within our lifetimes. We were still working to define a gene and to understand how genes were regulated, which was the subject that I worked on when I was young. The technology available was primitive. There was no cloning technology. I graduated from college and went on to get a PhD. Incredibly, by the time I received my PhD, it had become possible to apply molecular biology to study cancer.

I went into the cancer field because it had become possible to move molecular genetic methods into this important disease area. It became possible, in part, because animals are susceptible to cancer-causing viruses, and we realized that the same kind of methodology and thinking that we were applying so successfully to viruses that grew in bacteria could be applied to viruses that cause cancer in animals. The existence of cancer-causing viruses in animals, particularly retroviruses, propelled this science forward. I went into cancer research as a result. I worked on cancer-causing viruses for about 15 years, during which there were huge discoveries, particularly the discovery of oncogenes in retroviruses and their origin from cellular genes. The field exploded. It was very exciting. However, as I continued in cancer research, I found the cancer field to not be nearly as friendly to women scientists as the bacterial virus field had been.

How do you find a model animal in which you can study the genetics of behavior – is it possible?

Had you felt this way before, that the cancer field was unfriendly to women scientists, early in your career? How was it when you were working on the subject in the lab of the future Nobel Prize winner, Jim Watson?

Jim not only inspired me to become a scientist, he encouraged and advised me on how to become an independent scientist. Jim urged me go to graduate school and then he urged me to accept a job as a professor. Up until then, women were unlikely to become independent scientists and couldn't get university jobs. Few women continued after a PhD unless they married a powerful scientist or were otherwise able to get a long-term position in a professor's lab. But I had a different experience thanks to Jim Watson, so I really didn't think about gender discrimination. When I was very young I was accepted by my fellow students and promoted by Jim Watson and other young faculty at Harvard, particularly Mark Ptashne and Guido Guiodotti. I thought, 'Gosh, gender discrimination isn't my

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problem.' I wondered what those feminists were complaining about. But when I went to MIT, I left Jim Watson's sphere of influence, where he was so powerful that if you were in his good graces you would be fine. Jim took care of so many students. I was just one of many that he promoted. He picked out a lot of young people he wanted to promote and it was clear that if you were one of them you had a good chance of success. I just happened to be a woman. Now, I think that was even part of my good luck perhaps.

I once asked another young scientist at Harvard, 'Why did Jim pick me?' He said, 'We all want to know if a woman can make it. We think you could be the one.' It was so unexpected for a woman of my generation to pursue, much less succeed, in a research career that, without Jim's support, I know I couldn't have done it. When I left Jim's sphere of influence and went to MIT, I didn't have that kind of support. There were fabulous scientists and resources and wonderful people there too, but not that level of personal support which I had enjoyed as a student. It was very tough. I thought, 'I'm not aggressive enough for science, I'm not self-promoting enough. I was fine as long as I was with my friends at Harvard, but now I'm in less-supportive territory, starting out all over again.' Something was wrong but I didn't exactly know what it was.

I loved the science in that exciting era in the field of early molecular cancer virology, but I felt that the cancer field wasn't as much fun as time went by. I felt excluded and unable to become a full participant, even though I was uncertain of the reasons why. I began to suspect it was because I was a woman, but mostly I thought it was my own lack of aggressiveness or some other personal failing. I began to think of changing fields. I was very interested in the AIDS field and it was closely related to the kind of research I was doing. But I felt that women would have an even tougher time in the AIDS field because it was dominated by a small group of incredibly aggressive men who controlled everything. By then, I had learned that often these types of men did not accept women as full and equal participants, as real colleagues.

In light of my experience, you can imagine my incredible delight to hear that a woman won the Nobel Prize for discovering the AIDS virus: Françoise Barré-Sinoussi. It was particularly wonderful to me because it was partly the treatment of her that made me realize that I didn't want to go into the AIDS field. I felt that women would be treated as invisible, so it was a joy to see her get the credit she deserved. For years, two powerful men, one American, one French, fought over the credit and patents regarding the discovery of HIV, whereas the person who had made such essential contributions to finding the virus, Françoise Barré-Sinoussi, was seldom mentioned. Things like that just make you feel despair if you are a woman scientist: what's the point in being in a field where you are not a full participant or where your work isn't recognized for its value? It is demoralizing. It's almost as demoralizing watching other women being treated this way as when it is yourself. I saw this happen to so many women scientists around me, as well as having it happen to me. You learned to maneuver your way around the problem, but it was very difficult.

Another reason I changed fields was that I felt cancer research did not need me anymore in the way it had when it was risky. Oncogenes had been discovered now and we thought cancer research was on a very clear path. We thought that you had to study the oncogenes and see if you could find drugs that inhibited the action of these genes' products. There was reason to think that if you could, you would cure cancer. I figured if everyone knows what to do, they don't need me.

At this point, I thought about leaving cancer research. My other passion from the day that I had heard Jim lecture, was whether one could apply molecular biology and genetics to the brain to understand animal behavior. If cancer had become accessible to molecular biology so quickly, was it possible the same thing had happened to understanding the brain and behavior? I decided to check this out. I wanted to do forward genetic screens to study behavior and I wanted to do it in a vertebrate system.

I picked the zebrafish since this model was tractable for forward genetic screening. I heard that Christiane Nüsslein-Volhard, the great Drosophila researcher from Germany had started to work on zebrafish. I had read about her work but didn't know her. She was a great – really ***the*** great – developmental biologist of her era. I thought I could go to her lab and learn how to work with zebrafish and how to do large-scale screens, then go to a neurobiology lab and learn how to set up behavioral assays, and then do genetic screens for genes that affect behavior. So off I went to Germany to Janni's (nickname for Christiane, pronounced 'Yanni') lab to learn about zebrafish and large-scale genetic screens in vertebrate animals. Soon after I arrived, I could see that behavioral screens were too difficult in fish at the time. However, the fish was the ideal vertebrate for finding genes required for early developmental traits. I decided to develop methodology for doing forward genetic screens in zebrafish with the hope that, one day, the methods might be used for behavioral screens as well. My love for science returned because working with Janni was so much fun. It was a joy to be back in the lab. Over the next few years, back at MIT, with my incredibly talented students and postdocs, we were able to develop a really efficient method of insertional mutagenesis. This was a very risky project but it worked out. It was a wonderful time.

How did you originally choose the zebrafish as a model organism?

What I was really interested in was human behavior. Being a biologist I thought, 'How do you find a model animal in which you can study the genetics of behavior – is it possible?' I wanted to study a vertebrate system and at the time I thought there were two possibilities; one was to use mice, because Mario Capecchi and Oliver Smithies had just got knockout mice to work. Now you could make a mutation in a gene and ask if it affected the behavior of the mouse, which was a new technology. But I already knew from my work on retroviruses of mice that I didn't enjoy working with mice. The other approach I considered was to use human genetics, taking families and looking for linkage to genes. But these methods require a very large lab, huge resources and lots of money. I knew that women didn't usually have large labs and lots of money and I suspected they would have trouble getting these sorts of resources. (This was a case where bias against women worked in my favor, because this science was too hard and didn't advance at all in this era.) Then, I heard about the zebrafish and I thought it was perfect because it's a vertebrate animal in which you can do large-scale forward genetics, a technique I deeply believed in. Furthermore, it turned

out to be a joy to work with fish. In addition, the most successful scientist in developmental biology was using the zebrafish and she was a woman. So, I thought it's the one for me.

What was it like to secure funding while moving into a new area of science and away from your previous expertise?
Almost impossible. People told me I was crazy. I applied to the NIH and they wrote back and said this old lady (I was about 50) thinks she can change her field of research at her age? Well, you have to admire her courage, but it is crazy. Those were the comments, followed by a very low score and an unfunded grant of course.

How did you develop the traction needed to push into a new area?
It was very hard to make a switch, and probably very foolhardy in hindsight, but I really wanted to do it. I was very determined and very lucky. I got some gifts from a personal friend, Arthur Merrill. By matching funds through his company, he gave me US$30,000 a year for a number of years and this made it possible for me to begin in a new field. Then I got a foundation grant, thanks to two of my colleagues at MIT, Phil Sharp and the chairman of our department, Gene Brown. They knew what I was trying to do and decided to give me a boost by supporting my application for a US$50,000 grant from the Whitaker Foundation, a private foundation that gives money to MIT. Then I applied to the National Science Foundation (NSF). There were two wonderful women there, Judy Plessset and Delil Nasser, who came up to MIT to see if I was serious and really had some fish tanks. Thanks to them, I got a small grant from the NSF. I still couldn't get NIH funding.

Then a miracle occurred. We were making real progress and found that mouse retroviruses might be able to work as insertional mutagens in the fish, although only at very low frequency at first. Around this time, Amgen gave a great deal of money to MIT and faculty could apply for grants. So I applied for US$30,000 and I mentioned our progress with insertional mutagenesis. They called me and said they were very interested in this and were prepared to give me enough money to develop the technique, carry out a pilot screen and, if it worked, fund the majority of a large-scale screen. I couldn't believe my good luck. They did exactly what they said. In the end, they gave us about US$8 million. Furthermore, after the pilot screen worked, the NIH finally became enthusiastic and kicked in about one third of the cost of the large-scale screen. It was an incredibly exciting time. We didn't know for several years if it could work technically. When it did, we broke out the champagne.

When did you begin large-scale genetic screens in zebrafish?
It was about 17 years ago. It took several years for the transition. It was hard, but so much fun. So many exceptional young people wanted to work on zebrafish, so we had wonderful applicants to the lab. These people – initially Adam Amsterdam, Nick Gaiano, Shou Lin, Tina Yoon and then others – made the work possible. It was such a joy.

Now that you are generating all of these data, how do you decide where to focus?
This is a wonderful process. The people who came to work in the lab were interested in different types of mutants. When our big screen was finished, in which we looked for all mutants that produced a visible defect in early development, including cell viability, postdocs took many of the mutants with them to start their own careers. This was one of many benefits to this approach because the screen identifies more genes than one lab can possibly work on. I waited to see what was left that was interesting to me.

Other people in the zebrafish field had done similar screens and found and kept mutants that affected developmental processes, but they had discarded the mutants that primarily affected cell viability. In contrast, we kept all the mutants and, because we had used insertional mutagenesis instead of chemicals to make the mutants, we were able to rapidly clone the genes, including many genes necessary for cell viability. The technology was so good that, incredibly, almost all the genes were cloned by a single person – my remarkable student, by then a postdoc and now a research scientist, Dr Adam Amsterdam. So, we didn't throw anything out. We kept everything.

About 60% of the mutants are in genes that affect cell viability and, therefore, organism viability, but they're not really developmental in the sense that they are not so specific in terms of being needed to make a particular structure. The existence of these cell-essential mutants was extremely interesting in examining biological processes in the cell, such as DNA repair, and the cell cycle. It turned out that, when mutated, some of these genes predisposed the fish to cancer. A number of zebrafish lines that we generated were predisposed to get cancer. My passion for cancer research returned. So, in collaboration with a colleague, Professor Jackie Lees, I went back into the molecular biology of cancer using zebrafish. I discovered that, despite the enormous progress that had been made in basic cancer research since I left the field, many of the most important questions were still unanswered and, of course, most cancers have not yet been cured.

Do you feel that there's a difference in the cancer field having spent some time away from it?
The field is breathtaking. The progress is astonishing. But tough questions remain. When I left the field a big question was, 'Does it take mutations in three genes or five genes to make cancer in humans?' Now people are trying to figure out if it takes mutations in dozens or even in 100s of genes? It is still not known how many genes have to be mutated to make cancer, or how many genes have to be inhibited (i.e. drug-able), in order to control the growth of most cancers. We hoped it would be simpler and although the progress has been spectacular, it's still not enough.

Another interesting thing to ask is whether the field had advanced in terms of the progress of women? The answer seems to be yes and no, depending on the specific area of the field. For example, I got a note this morning about a cancer meeting in Boston. It looks like a fabulous meeting and there are 31 speakers, but only three are women. This is astonishing when you consider that the majority of undergrad majors in biology are women, 50% of medical students are women, and women get about half the biology PhDs. This suggests to me that women are still not full participants in some areas of cancer research.

What's amazing is that some women, such as you, managed to create a successful career anyway.
We were the lucky ones. In some cases I think we were also the ones who were in

denial about bias, which may have helped us persist in the beginning We were also people who loved science so much we couldn't really imagine life without it. People look at successful women and say, 'Well, so and so made it. What's wrong with you?' That's not really a good argument. For too many women, the playing field is still not level and that is unacceptable. This is unnecessary now, which was not true when I began. Then we didn't understand the obstacles women faced, now we do, and we know how to fix most of them.

There are certainly some extraordinary changes, and much progress, for women in science and society; I don't mean to be too discouraging. In biology, for the first 25 years that I was at MIT, there were 15% women faculty in my department and today it's between 20-25% and, most important, women hold powerful administrative positions. That's a big difference. The percentage of women on the faculty in the whole School of Science at MIT never rose above 8% for my first 20 years at MIT, but today it's 17% (the same as the percentage of women in the US Senate by the way!). There are many women in the administration and there are powerful women presidents of MIT, Princeton, Harvard and many other research universities, which was unimaginable when I was young. We need these women to lead visibly and to support the institutional changes that will level the playing field so that young women know there is a voice for them and so they don't have to keep fighting these same old battles. This is what it takes to create a friendly environment for everyone.

You have identified many roadblocks in the academic system. Do you have advice for helping young people navigate through the tenure structure?

It really is different now relative to when I first started. There really are more women and, most important, some powerful women. These women who made it through are trying to change the system so it will be different for future generations of women, and this is working. In my generation, many, if not most, women in this career path thought that they were not able to have children and a high-powered career because there was no help unless you had parents, or someone else, to help you, or a lot of money. Universities now have family leave policies that make it more possible to have a family, and these policies also attempt to take the stigma out of women having maternity leave. These things just didn't exist when I started. So, the system is changing by having women move up through the ranks and responding to the needs they encounter.

Young women should go into science believing they can do everything and when they hit an obstacle they should seek out women who have succeeded and ask how they did it. They need both role models and mentors. A mentor can be a man or a woman. You need powerful people who understand the system and know how to succeed in it. When I was young, I had to have a Jim Watson since there weren't any powerful women. Today, there are powerful women role models and both powerful men and women who can encourage and mentor young women who are interested in science.

You already mentioned cancer and neurobiology as fields that you find interesting. Do you see one of those as holding the big question for medical science in the near future?

There has been a lot of progress in cancer, although it's far from solved, and some of the treatments and cures are still pretty brutal. There is a lot of work to be done and we've got to press on at full speed. This is still a passion of mine.

What also intrigues me, 45 years after hearing Jim Watson give that lecture is, 'How does the brain work and how does it solve problems?' Perhaps the thing that interests me the most is, 'Can we understand human behavior, in all its complexity, through biology?' Ultimately, I am interested in how human behavior could explain social systems and the things that go wrong in society. Why do we have war, prejudice and such? If we understood enough about human behavior, could we do something about it? Could you make a pill to prevent war? That's a really important question!

I realized, ironically, that I learned far more about the brain and human behavior from studying gender discrimination than I did from studying molecular biology. Then, I discovered that psychologists already knew a lot about what I had discovered by my first-hand experiences and by studying these issues myself. Psychologists have learned a lot about behavior, including bias. I'm very excited because I think that work on gender bias and race bias by psychologists is phenomenal. Could we ever understand bias at a biological level? I don't know. Psychologists now think that you begin to learn cultural beliefs, including about gender, at between 3 and 9 months of age. No matter how many trucks you give to the little girls, and how many dolls you give to the little boys, they're still going to figure out how society really works. I think that such understanding might be one of the most important contributions we could make to human society. If I were younger, I would think about going in this direction. Whether biology will be able to understand the psychologists' observations and, if so, how long from now, I can't imagine.

I'm 65 and I'm just as intrigued with genetics and molecular biology as the day I sat in Jim Watson's lecture in 1963. I'm still passionately interested in seeing cancer cured and I expect to live long enough to see better prevention, early detection, control and even cures of cancer. As for the brain, I got to work on it by accident and am only sad that I can't have another entire career in science to follow up this second passion. Understanding the biological basis for human behavior, including discrimination, drove me when I was young. I wanted to understand diseases and be able to cure them and I wanted to understand why the world was a screwed-up place and fix it. I still believe that science, including biology and psychology, can help accomplish both of these goals.

DMM greatly appreciates Nancy Hopkin's willingness to share her personal experiences that have shaped her unique and successful career. She overcame obstacles to advance scientific social and technical practices and has defined novel purposes for a variety of genes. We are grateful for the opportunity to present her story here as A Model for Life.

Nancy Hopkins was interviewed by Kristin Kain, Associate Reviews Editor for DMM. This piece has been edited and condensed with approval from the interviewee.